POLIOMYELITIS AS A COMPLEX INFECTION*

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Poliomyelitis and Coxsackie virus infections are characteristically closely associated in time and place and are similar in many respects. Whatever mutual effects the two may have can therefore be expected at times to modify the course of the natural disease. Early observations suggested sharp differences in this respect between the Group A and Group B Coxsackie viruses. The former were frequently associated with paralytic poliomyelitis, the Group B outbreaks were notable for the absence of paralysis. Recognition of an interference between Group B and polioviruses has provided an explanation of the second observation but little experimental evidence was found to indicate that Group A infections intensify the effects of poliovirus infection (1–6).

The present experiments indicate that under certain circumstances at least one Group A virus is capable of exaggerating the paralytic effect of a poliovirus infection. The work was undertaken when a Group A virus became available that induces poliomyelitis-like lesions in monkeys (7). It was suspected that earlier failures may have been due to the use of virus types (or strains) that lacked or had lost neuropathogenicity and that an enhancing effect of combined infection might represent the simple addition of two similar morbid processes.

Materials and Methods

Viruses. The more pertinent features of the strains used have been summarized in Table I. The attenuated strain of Type 1 poliovirus was provided by Dr. Albert B. Sabin who had found that it caused neuronal degeneration but not paralysis following the intracerebral inoculation of monkeys (8–10). The strain was maintained by passage in monkey kidney cell cultures.

The Coxsackie A-14 strain was sent to us by Dr. James Gear. It has since been adapted to adult mice in which it induces poliomyelitis-like lesions. The adult mouse-adapted strain also induces lesions of the motor neurons in monkeys inoculated intracerebrally but none of the animals has been paralyzed (7).

The AB IV virus is a strain of Group A, Type 7, Coxsackie virus that was supplied to us by Dr. Karl Habel who had received the 24th newborn mouse passage from Russia and had made

* A number of the monkeys were a gift of The National Foundation.

‡ Present address: The National Foundation, New York.

5 additional transfers before sending it to us (11). It causes poliomyelitis-like lesions in monkeys and at times tremors and paralysis as well (12).

The viruses were maintained and suspensions prepared by methods described in detail elsewhere (13). Suspensions were stored at −20°C. and were thawed but once.

Monkeys. Young, healthy *cynomolgus* monkeys between 1200 and 1800 gm. in weight were used whenever possible. The intracerebral inoculations were made during light anaesthesia.

TABLE I

Properties of Virus Strains Used

Strain	Human source	Place of origin	Passages*	Usual titer of pools
Polio 1 "80-4" No. 5733‡	Feces	Cleveland	6 on monkey kidney cells	$10^{-6.8}$
Coxsackie A-14 No. 52113	Feces	So. Africa	27 adult mice 11 baby mice	10^{-3} $10^{-7.2}$
NIH AB IV No. 56135	Feces	Russia	9 baby mice	$10^{-5.8}$

* Passages after receipt in this laboratory.

‡ Culture collection number.

TABLE II

*Description and Definition of Grades of Paralysis**

Grade	Description and Definition
5	"Normal"
4	Definite weakness but capable of using limb effectively in climbing, running, or jumping
3	Uses limb poorly in climbing, running, or jumping
2	Barely able to use limb in climbing or running movements but able to move limb segments readily against gravity
1	Dangling limb with only feeble movements at skeletal joints
0	Complete flaccid paralysis or only faint traces of movement

* See reference 14.

The animals were inspected daily. Rectal temperatures were taken in the morning before feeding and again if the animal was not caught at once or was excited. All of the animals had been afebrile for 3 to 4 days before being used. During periods of illness, the temperature readings were repeated in the late afternoon. Muscle function was tested by observation of the animals running, climbing, and jumping, and by comparing the strength of the extremities and the patellar reflexes. Loss of muscle power and paralysis were graded as proposed by Bodian (14) (Table II). Atrophy was noted when present.

Histologic examination of the spinal cords was limited to two levels, C_5-Th_1 and L_1-S_3. The remaining tissue was frozen as were portions of the medullae and used for the re-isolation of the viruses. Since the possibility of isolation is optimal only during the first days after onset of illness (15–17), no isolation was attempted with monkeys to be sacrificed later. The suspen-

sions of monkey tissues were inoculated on monkey kidney cell cultures and injected into 1- to 3-day-old mice.

EXPERIMENTAL

Preliminary Tests. To confirm the earlier observations of the pathogenicity of the strains (7–10), suspensions of the Coxsackie A-14 and the polio 1 viruses were inoculated into two groups each of *cynomolgus* monkeys of the size we had chosen for the experiments (Fig. 1). In two of the groups, the inoculations

TABLE III

Response of Monkeys to Test Viruses

No. of monkeys tested	Strain	Reinjection	Intervals	Clinical response			Histol. exam. Lesions*
				Paralysis	Fever	None	
4	A-14	—	—	—	3	1	c moderate l "
3	A-14	A-14	On days 2 and 3	—	3	—	c moderate l "
3	"80-4"	—	—	—	2	1	c slight to moderate l slight to moderate
4	"80-4"	"80-4"	5 days	—	4	—	c slight to moderate l slight to moderate

* c = cervical cord C_5-Th_1.
l = lumbar cord L_1-S_3.

were repeated to exclude the possibility that re-injection would of itself cause more severe disease (18, 19). The results are summarized in Table III.

The first group of 4 monkeys was injected intracerebrally (0.2 ml.) and intramuscularly (0.1 ml. in the calf) using a 10 per cent suspension (10^2PD_{50}) of pooled infected adult mouse brain and cord. Three of the animals responded with fever between 3 and 5 days postinoculation and were sacrificed on the 6th day. Histologic examination disclosed moderately severe lesions of the anterior horns of the spinal cords. The A-14 virus was readily re-isolated in baby mice. The 4th monkey showed no clinical response and, when sacrificed on the 22nd day, scanty lesions in the stage of recovery were found. Virus isolation was not attempted.

In the second group, 3 monkeys were injected by the same routes using a 10 per cent leg suspension of infected baby mice ($10^{6.2}PD_{50}$) as inoculum. In addition, 0.4 ml. of the suspension was injected subcutaneously on the 2nd and 3rd days. Slight fever was the only evidence of illness. The lesions in the spinal cord were similar to those in the first group and in early experiments (7).

The Type 1 poliovirus was first tested in 3 monkeys by inoculating 0.5 ml. ($10^{6.2}TCD_{50}$) intracerebrally. They were sacrificed on the 10th day when 2 were febrile. Slight to moderate

lesions were found in their spinal cords largely in the cervical segments. The virus was recovered from medulla-cord suspensions of 2 but not the 3rd.

Following 6 passages in trypsinized monkey kidney tissue cultures, 0.5 ml. ($10^{5.8}TCD_{50}$) of the culture fluid was injected intracerebrally into 4 monkeys. The injection was repeated 5 days later using the same amount of virus but the opposite hemisphere. All the animals responded with fever. No muscle weakness or paralysis was found. Slight to moderate destruc-

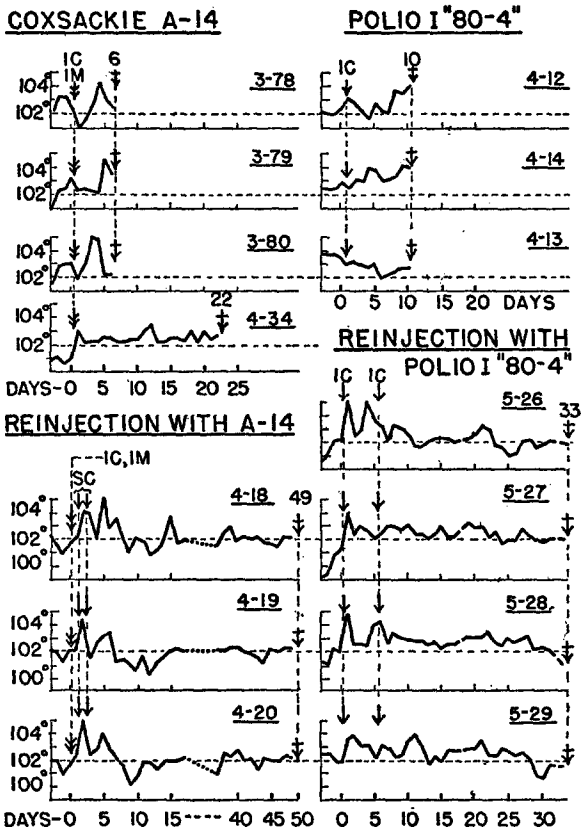

FIG. 1. Clinical response of *cynomolgus* monkeys to test viruses

tion in the anterior horn cells could be seen in the histologic specimens of all 4 monkeys. Since they were sacrificed 33 days after inoculation, virus identification was not undertaken.

Type 1 Polio and Coxsackie A-14 Infections. Several experiments have been performed using the polio 1 and the Coxsackie A-14 viruses simultaneously or at various intervals (Table IV). Additional controls were included in all of the experiments.

The Coxsackie controls received 0.2 ml. intracerebrally and 0.1 ml. intramuscularly (leg) of a 10 per cent suspension ($10^{2}PD_{50}$) of infected adult mice brain and cord. The polio controls

were given 0.5 ml. of the 10 per cent dilution of the tissue culture fluid ($10^{5.8}TCD_{50}$) intracerebrally. To determine whether a second intracerebral or intramuscular trauma might provoke or exaggerate the response to the poliovirus (18–21), 5 of these were re-inoculated intracerebrally (0.2 ml.) and intramuscularly (0.1 ml.) with a 10 per cent suspension of normal adult mouse brain and cord 5 days later (Table IV). Fever was the only clinical response. No evidence of weakness or paralysis was found. The clinical and histopathologic picture was similar to that seen in the preliminary tests.

Simultaneous Inoculations. Two groups of monkeys received the A-14 and polio 1 viruses simultaneously. In 3, a suspension of infected baby mouse leg

TABLE IV

Clinical Response to Injections Given at Various Intervals

Polio 1 "80-4" + Coxsackie A-14.

	Test No.	No. of monkeys tested	Clinical response			Interval between injections
			Paralysis	Fever	None	
A-14 controls	2B	2	—	1	1	—
	2C	2	—	1	1	—
	2D	1	—	1	—	—
Polio controls	2B	3	—	1	2	—
	2C	3*	—	—	3	5 days
	2D	2*	—	2	—	5 "
Test animals	2A	5	—	2	3	Simultaneously
	2B	5	4	1	1	5 days
	2C	5	2	2	2	5 "
	2D	8	6	7	—	5 "
	2B	3‡	2	2	—	25 "

* Received a 2nd injection i.c./i.m. of normal mouse brain suspension after 5 days.

‡ Monkeys responded exclusively after the 2nd injection.

was used, in 2, a suspension of pooled adult mouse brain and cord. The responses are shown in Table IV (test 2A) and in Fig. 2. Paralysis did not appear. The animals that had received the adult mouse suspension did, however, show somewhat more severe lesions. The group of 3 were sacrificed on the 10th day and the A-14 virus was isolated from a suspension of spinal cord of one of these.

Inoculation at 5 Day Intervals. Three groups (18 monkeys) were injected with the Type 1 poliovirus and 5 days later with the Coxsackie A-14 virus using infected adult mouse tissue in the amounts and by the routes employed for the controls (Table IV).

In the 1st group (test 2B), 3 animals responded dramatically 3 to 5 days after the 2nd injection. Tremor appeared in the extremities followed by progressive paralysis. The temperatures became subnormal and within the day all

3 were moribund (Fig. 2). The 4th monkey had fever on the 5th day following the 2nd injection and the right patellar reflex disappeared. Two days later the leg was paralyzed and within 2 weeks showed severe muscle atrophy. Before the monkey was sacrificed on the 82nd day, the animal was able to use the leg slightly and the patellar reflex had returned. The atrophy was as severe as

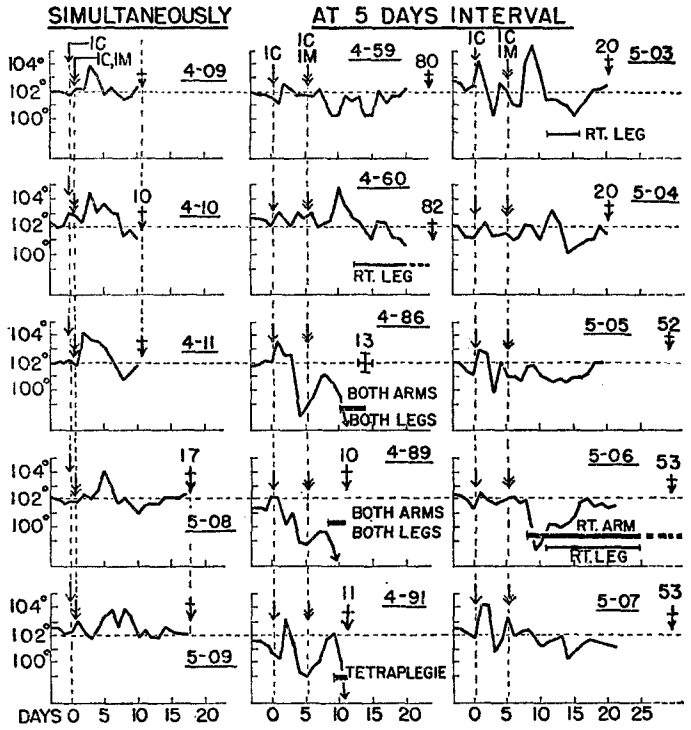

FIG. 2. Temperature charts of *cynomolgus* monkeys injected with Polio 1 "80-4" and Coxsackie A-14.

before. Only one of the 5 monkeys escaped paralysis. The severity of the paralysis in the 4 corresponded with the histopathologic findings (Table V). Few motor neurons could be found in the 3 most severe cases. Both viruses were isolated from the spinal cords of these 3 animals. The other 2 were not tested.

In the 2nd group, 2 monkeys responded with paralysis. One of them (Nc. 5-03) became febrile on days 3 and 4 after the Coxsackie virus inoculation. The right patellar reflex vanished at the same time. Two days later paresis appeared in the right leg and was conspicuous during the next 5 days. Then the limb improved gradually and before the monkey was sacrificed, the patellar reflex was again present. The 2nd animal (No. 5-06) showed onset of paralysis

TABLE V

Grades of Paralysis and Spinal Cord Lesions

Polio 1 "80-4" + Coxsackie A-14.

Test No.	Monkey No.	Fever*	Maximal‡ paralysis	†§	¥‖	Histological examination Lesions	
						Cerv. cord C_5-Th_1	Lumb. cord L_1-S_3
2A	4-09	3	—	—	10	Moderate	Moderate
Simultaneous injection	4-10	3–4	—	—	10	None	"
	4-11	2–3	—	—	10	"	"
	5-08	5	—	—	17	Moderate	"
	5-09	6–8	—	—	17	"	"
2B	4-59	—	—	—	80	None	Slight
5 days interval	4-60	10–11	55/52	—	82	"	Moderate
	4-86	—	22/00	13	—	Extreme	Extreme
	4-89	—	11/22	—	10	"	Moderate
	4-91	—	00/00	—	11	"	Extreme
2C	5-03	8–9	55/53	—	20	Slight	Severe
5 days interval	5-04	4	—	—	20	None	Moderate
	5-05	—	—	—	52	"	Slight
	5-06	—	51/53	—	53	Severe	"
	5-07	—	—	—	53	None	"
2D	5-30	9–10	—	—	22	Slight	Slight
5 days interval	5-31	8–9	55/53	—	22	None	Moderate
	5-32	12	52/55	—	22	Moderate	Slight
	5-33	8–9	—	—	22	Slight	"
	5-34	9–10	52/45	—	22	Moderate	Moderate
	5-35	—	55/35	—	23	Slight	"
	5-36	7–8	55/25	—	23	"	"
	5-37	8–11/14	35/35	—	23	Moderate	"
2B	4-56	7–8/10–11	55/33	—	46	Moderate	Moderate
25 day interval	4-82	10–12	—	—	46	"	"
	4-84	—	11/11	32	—	Severe	Severe

* On days after first inoculation except response of Test 2B which occurred exclusively after second inoculation.

‡ Grades of paralysis shown in numbers for each limb: left arm right arm/left leg right leg; no grades given when no paralysis.

§ Died on day after 1st inoculation.

‖ Sacrificed on day after 1st inoculation.

in the right arm on the 3rd day. The temperature became subnormal, the right leg became weak and the right patellar reflex disappeared. Paralysis persisted for 14 days. The leg was still weak after 53 days. The paralysis in the arm was complete and was accompanied by severe muscle atrophy. Three monkeys

showed no paralysis. The clinical findings were again verified by histologic examination of the spinal cords (Table V). Virus isolations were not attempted.

Eight animals were included in the 3rd group. Six were paralyzed. In 5, the paralysis lasted from 4 to 11 days (Fig. 3). In 4, only one limb was involved. Paralysis was again preceded by fever, between the 2nd and 6th days, and in 3 cases by tremor. Paralysis of the legs was preceded by the loss of the patellar reflex. In one monkey (No. 5-36), the reflexes of both legs disappeared but the

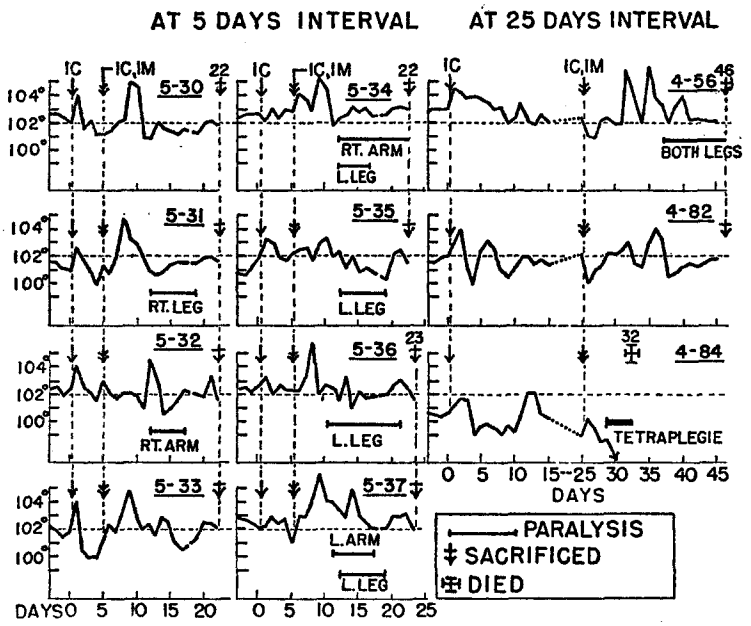

FIG. 3. Temperature charts of *cynomolgus* monkeys injected with Polio 1 "80-4" and Coxsackie A-14.

paralysis was seen in only one leg. When this animal was sacrificed, slight muscle atrophy was found in the left leg.

Inoculations at 25 Day Intervals. Three monkeys were injected with the same inocula, using the amounts and routes of the previous experiment. They first received the poliovirus and 25 days later the Coxsackie virus (Fig. 3). The first animal had 2 severe febrile episodes following the 2nd injection. The sequences of tremor (in both legs), loss of patellar reflexes, and onset of paralysis (bilateral) developed as before. The 2nd animal showed only fever. The 3rd was severely affected. The temperatures dropped after the 2nd injection and on the 5th day paralysis appeared and involved all of the extremities 2 days later. The Coxsackie virus was readily isolated from a suspension of spinal

cord. Moderate to severe lesions were found in the histologic preparations that corresponded well with the clinical signs of disease in these animals.

Type 1 Polio and Coxsackie A-7 Infections. To test the combination of the Type 1 polio and the AB IV strain of A-7, 10 monkeys were used, of which 5 served as controls (Table VI). Two of the controls and 1 test animal were young *rhesus* (*Macaca mulatta*) monkeys. The poliovirus was given as before. The A-7 virus, passed in baby mice, was inoculated intramuscularly (0.5 ml. of 10 per cent leg suspension having a titer in baby mice of $10^{4.8}PD_{50}$).

TABLE VI

Response to Injections Given at a 5 Day Interval

Polio 1 "80-4" + AB IV.

	Monkey No.	Clinical response		♆*	Histological examination Lesions	
		Max. paralysis	Fever		Cerv. cord C_5-Th_1	Lumb. cord L_1-S_3
AB IV controls	4-35‡	—	4–5 7–8	22	Severe	Severe
	4-37	—	5–6	13	Moderate	Moderate
	4-44	—	4–6	33	None	Slight
Polio controls	4-36‡	—	11	22	Moderate	Slight
	4-45	—	—	33	"	Moderate
Test animals	4-38‡	51/55	—	19	Extreme	None
	4-41	—	9–11	34	Slight	Slight
	4-49	—	10	34	"	"
	4-50	—	—	34	"	None
	4-52	55/51	7-8	34	None	Extreme

* Sacrificed on day after 1st inoculation.

‡ Rhesus monkeys.

The polio controls responded as previously and their lesions were as expected. The A-7 controls became febrile between the 4th and 6th days and one (No. 4-35) again on the 7th and 8th days. An attempt to isolate the virus from the spinal cord of monkey No. 4-37 (sacrificed on the 13th day) failed. Of the 5 test animals, 2 became paralyzed. The histologic examination of their spinal cords revealed more severe lesions than those caused by the poliovirus or the A-7 strain alone.

DISCUSSION

Little is known of the nature of human infections with the A-14 Coxsackie virus used in the present experiments but A-1, which shares with A-14 the

ability to attack the motor neurons in the spinal cords of adult mice, has not infrequently been associated with poliovirus in instances of severe paralytic disease. Indeed the 2 boys from whom the original strains were isolated were both paralyzed and Coxsackie A-1, as well as Type 1 poliovirus, were isolated from their feces. Other strains were recovered in 1949 from outbreaks of unusually severe illnesses and the same type was prevalent in Easton, Pennsylvania, during the remarkable epidemic studied by Melnick and Kaplan (22) in which the frequency and severity of paralysis were noteworthy.

Whether the present experiments are a copy of such natural phenomena, we do not know, because the experiments involved an unnatural route of infection. This is especially worrisome because it has been recognized for many years that repeated intracerebral inoculation of poliovirus suspensions may accelerate paralytic disease (18). This was not true of our double inoculated controls. Presumably the outcome in all such experiments owes a great deal to the properties of the strains of virus used and we deliberately sought, in the present work, to measure the results of combined infection with strains that had never caused paralysis when given alone. One experiment has been done using a peripherally paralytogenic strain of Type 1 poliovirus but the outcome, in the controls, was too unpredictable to justify further trials. It may be noted, however, that combined infection under these conditions was also more severe than among the control animals.

Nevertheless the experiments seem interesting in that they suggest the possibility that paralytic poliomyelitis may at times be a complex infection and that simultaneous infection with polio and polio-like viruses, each of which causes disease of the motor mechanism, and which do not interfere, may, by simple summation of the two processes, result in more severe paralysis than either alone is capable of causing. We have speculated whether repeated attacks of nonparalytic disease might culminate in paralysis because each had destroyed a number of motor cells, cells which are never replaced. Some of the unexplained variations of the severity of paralysis in individuals and in epidemics may, at times, reflect such combined or cumulative effects.

Whatever the technical defects in the present experiments, it seems reasonably well established that neither the trauma of a 2nd intracerebral inoculation by itself or a 2nd intracerebral inoculation of the attenuated poliovirus used was capable of causing paralysis under the present conditions. It is most unlikely that the paralysis observed in the experimental groups was due to chance. An outcome showing a contrast this great, as in the polio 1 A-14 experiments, would occur by chance alone in fewer than 1 in 500 trials.

Careful attention should be given to the infection of children with attenuated polio viruses during times when Group A virus infection might be expected to be prevalent. Whether this represents a real danger is unknown. We know of no reports suggesting that it has ever led to paralytic disease but the possibility

deserves consideration. Presumably the danger could be avoided by vaccinating with attenuated strains of polioviruses at times when these other viruses do not occur.

Finally, we would like to remark on the opposite effect of Group B viruses and to call attention to the fact that the three types of polioviruses interfere with one another. It would seem that such relationships between the enteric viruses deserve closer attention. Many of the ambiguities of the epidemiology of paralytic poliomyelitis might seem less mystifying if we knew more of the ecology of the enteroviruses (23–26).

SUMMARY

Young *cynomolgus* monkeys inoculated intracerebrally with an attenuated Type 1 polio virus and, after 5 days, with a monkey adapted Coxsackie A-14 virus frequently became paralyzed. Neither virus alone was capable of inducing paralysis.

Similar results were observed when the AB IV strain of Coxsackie A-7 was substituted for the A-14 virus. In this case the 2nd inoculation was made intramuscularly.

Paralytic poliomyelitis may at times represent the summation of two infections, the total of motor neuron destruction by two independent and non-interfering enteroviruses.

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